Intensifying technologies to increase monoclonal antibody upstream process productivity

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Abstract

This work demonstrates the potential of upstream process optimization and intensification strategies that can be used to increase monoclonal antibody (mAb) upstream process productivity while reducing manufacturing costs. The Thermo Fisher Scientific technologies highlighted in this study increased upstream mAb product yield from the industry expectation of 4 g/L to as much as 8.4 g/L. These intensification strategies can also help increase manufacturing speed and output while reducing labor and consumable costs.

Introduction

Monoclonal antibody production has been revolutionizing healthcare by providing targeted therapies for a wide range of diseases, such as cancer, autoimmune disorders, and inflammatory conditions. Increasing productivity in mAb manufacturing can support accelerated time-to-market and reduced costs for these life-saving treatments. Upstream process development and intensification strategies can accelerate the manufacturing workflow by enabling higher yields and greater cost efficiency. Key approaches, such as generating high-producing cell lines and high-density cell banks (HDCBs) along with perfusion and intensified fed-batch bioreactor processes, can help enhance cell growth and productivity, streamline process operations, and support consistent product quality. Integrating these strategies significantly intensifies the upstream process, thereby improving scalability, reducing production costs, and ultimately boosting the overall efficiency of mAb production. The synergy created by combining high-producing cell lines, high-performing media, optimized feed strategies, and intensified bioreactor processes further elevates productivity and operational efficiency. This holistic approach allows each technology to complement the others, supporting a highly efficient workflow for mAb production intensification (Figure 1).

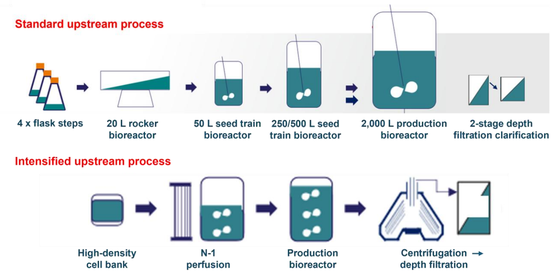

Figure 1. Standard upstream process vs. an intensified upstream process.

Materials and methods

Generation of a high-density cell bank

The Gibco™ CHOvantage™ cell line was used in this work. High-density working cell banks were created using the 50 L Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) in perfusion mode with Gibco™ High-Intensity Perfusion (HIP) CHO Medium. Banks were prepared in 50 mL bioprocess containers (BPCs) with 35 mL fill volumes at 50 x 10^6 cells/mL.

Inclusion of N-1 perfusion and intensified fed-batch cultures

This study used Gibco™ Efficient-Pro™ Medium, Gibco™ Efficient-Pro™ Feed 3, and Gibco™ Efficient-Pro™ Feed Enhancer. The HDCB was thawed and inoculated directly into the 50 L DynaDrive S.U.B. at a low operating volume to initiate the N-2 seed step. Once a specified viable cell volume (VCV) was reached based on capacitance, an automated medium and feed top-off step was performed to start the N-1 process. At another specified capacitance reading, perfusion was started automatically in the N-1 reactor. The perfusion rate was based on capacitance monitoring until a specified VCV was reached, and an automated high-VCD inoculum transfer to the N-stage 500 L Thermo Scientific™ DynaDrive™ Single-Use Bioreactor was initiated. The production bioreactor was fed using a capacitance-based feeding strategy. The culture was terminated on day 14, and the Thermo Scientific™ DynaSpin™ Single-Use Centrifuge was used for harvest and primary clarification.

Economic modeling data

Economic modeling data were generated using BioSolve Process™ modeling software. Assumptions of standard downstream processing remained the same for all economic modeling calculations.

Technologies used in the standard fed-batch process and the intensified fed-batch process

The standard fed-batch process consisted of 1) a standard vial working cell bank; 2) a seed train consisting of ~4 expansion steps in shake flasks and N-2 and N-1 expansion steps in the 50 L DynaDrive S.U.B.; and 3) a production step using a standard fed batch with standard VCD inoculum.

The intensified fed-batch process consisted of 1) a high-density cell bank; 2) a seed train consisting of an N-2 recovery step and an N-1 perfusion step in the 50 L DynaDrive S.U.B.; and 3) a production step using an intensified fed batch with high-VCD inoculum.

Results

Improved speed and process efficiency with high-density cell banks

Generation of a high-density working cell bank began with a master cell bank (MCB) that was scaled up via passaging before being transferred to the 50 L DynaDrive S.U.B., where perfusion culture was employed to increase cell density. The perfusion process allowed the cell density to rise above the 45 x 10^6 cells/mL required for high-density cell banking while maintaining the exponential growth needed for cell banking. An automated fill system was utilized to speed up the process of filling and freezing, which was critical for maintaining cell viability.

HDCBs produced with the DynaDrive S.U.B. are robust and comparable to standard cell lines in terms of performance. In this study, utilization of a HDCB prepared using the 50 L DynaDrive S.U.B. reduced the duration of the seed train stage from 18 days to 9 days as well as the number of FTE operators and space required for production (Table 1).

Table 1. Impact of high-density cell banking on upstream mAb production.

	Standard process	High-density cell bank
Process area (m^2)	957	721
Number of USP operators	14	5
Seed train duration	18 days	9 days

Optimized efficiency and sustainability with single-use systems

The use of single-use systems like bioreactors and centrifuges reduces the manufacturing footprint of mAb production by eliminating the need for extensive cleaning and sterilization, thus reducing downtime and utility requirements. These compact and flexible systems enhance space efficiency and operational flexibility, allowing a more efficient and scalable manufacturing process. The DynaSpin Single-Use Centrifuge efficiently clarifies high-density processes during the harvest step, achieving over 90% cell clearance and reducing filter usage by 70% relative to conventional depth filtration (Table 2). This facilitates reduction of pool volume, labor hours, equipment footprint, and buffer and water consumption, thereby enhancing the sustainability and efficiency of the manufacturing process.

Table 2. Impact of the DynaSpin Single-Use Centrifuge vs. depth filtration on mAb production.

	Depth filtration (standard process)	DynaSpin centrifuge harvest process	% Improvement
Filter capacity (L/m^2)	60	150	150%
Pool volume (L)	550	496	10%
Water usage (L)	1,980	550	20%
Total labor hours	25	4	76%
Equipment footprint (m^2)	17	4	72%
Depth filter usage	18 filters	5 filters	
Depth filter type	2-stage	Single-stage	

Including the DynaSpin Single-Use Centrifuge in the harvest step can increase filter capacity by 2.5-fold and reduce consumable cost and total labor hours. The DynaSpin centrifuge can also reduce depth filter usage by 70%, enabling a smaller warehouse footprint.

Enhancing upstream yield with an intensified process using the CHOvantage cell line and Gibco™ media

High-density cell banking reduces early seed train operations, which are among the most labor-intensive parts of upstream biomanufacturing since they require skilled operators and process control monitoring. Reducing the number of early seed operations can reduce labor expenses while simplifying manual upstream steps with automatable closed bioreactor operations.

The high turndown ratios of the 50 L (10:1) and 500 L (20:1) DynaDrive S.U.B.s allow low operating volumes. This reduces the time and number of vessels needed for the seed process, leading to more efficient resource utilization and consistent product quality. The DynaDrive S.U.B. turndown ratios enable small volume inoculum, N-2 and N-1 steps to be carried out in a single vessel, and transfer of high-VCD inoculum directly from the 50 L vessel to the 500 L vessel.

When N-1 perfusion and intensified fed-batch culture are used together, they have a synergistic effect. N-1 perfusion supports a high-density, healthy cell culture that can be rapidly scaled up in the production bioreactor. Intensified fed-batch culture enables the high-VCD inoculum to be expanded and maintained under optimal conditions, leading to more efficient and productive upstream processes.

In this study, combining high-density cell banking with N-2 and N-1 perfusion in a single-use bioreactor system and intensified fed-batch culturing increased the productivity of the upstream process from 3.2 g/L to 4.3 g/L. By incorporating the optimized mAb-producing CHOvantage cell line and Efficient-Pro Medium and Feed into the intensified process, the yield was further increased from 4.3 g/L to 8.4 g/L (Figure 2).

Figure 2. Impact of the high-production CHOvantage cell line and the intensified process on the yield of mAb production.

Addition of the high-production CHOvantage cell line to the intensified process significantly increased VCD and titer. Utilizing the CHOvantage cell line, Efficient-Pro Medium, Efficient-Pro Feed 3, and Efficient-Pro Feed Enhancer with an already intensified process increased specific productivity. Ultimately, the titer increased nearly 2-fold from 4.3 g/L to 8.4 g/L when compared to the intensified process, and the maximum VCD was reduced from >40 x 10^6 cells/mL to 30 x 10^6 cells/mL (Table 3).

Table 3. Impact of adding the high-production CHOvantage cell line and optimized medium and feed to the intensified process on mAb production efficiency.

	Standard process	Intensified process	Intensified process with the CHOvantage cell line
Cell titer	3.2 g/L	4.3 g/L	8.4 g/L
Kg per year	460.6	622.7	1,216.5
Cost per gram	$247.10	$202.90	$115.80
Estimated profit*	$24.4M	$60.5M	$224.1M

* Modeling assumes a mAb sale price of $300/g. Profit is dependent upon annual manufacturing output and the actual mAb sales price.

The combination of the CHOvantage cell line and optimized medium with the already intensified process increased the manufacturing yield by 110%. This increase in yield would translate to more kilograms produced per year (460.6 to 1,216.5), ultimately leading to an 800% improvement in profit ($24.4M to $224.1M).

Key findings

The combined strategies discussed herein hold the potential to:

- **Increase cell titer** by **163%** from 3.2 g/L to **8.4 g/L**
- **Increase kg/yr** by **2.6-fold** from **460.6** to **1,216.50**
- **Reduce cost per gram** by **50%** from **$247.10** to **$115.80**
- **Increase profit** by **800%** from **$24.4M to $224.1M**

Conclusions

Integrating the CHOvantage cell line, a high-performance medium, high-density cell banking, N-1 perfusion, intensified fed-batch culturing, and single-use bioreactor and centrifugation systems can enhance mAb production efficiency.

The scalability of this intensified process with the CHOvantage cell line supports easy transition from small-scale to large-scale production, making it a cost-effective solution for biomanufacturing. It can also facilitate decisions regarding annual output and assist in decisions regarding future manufacturing capability. Overall, these innovations help streamline the production process, reduce resource consumption, and maintain consistent product quality, positioning them as key drivers for future advancements in mAb manufacturing.

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