White paper

Monoclonal antibodies

Optimizing monoclonal antibody production: high-yield upstream process intensification strategies

Introduction

Monoclonal antibody (mAb) production has transformed healthcare by enabling targeted treatment of a wide range of diseases, including various types of cancer, autoimmune disease, and infectious disease. Increasing productivity in mAb manufacturing is crucial for shortening development timelines and generating drug products in sufficient quantities to deliver these life-saving immunotherapies. Upstream process development and intensification strategies can speed up the manufacturing workflow, increase mAb yield, and reduce operational and consumable costs. The use of high-production cell lines, high-density cell banks (HDCBs), and single-use systems together with intensified perfusion and fed-batch bioreactor processes is key. This approach promotes cell growth, enhances productivity, streamlines operations, and helps support consistent product quality.

Integrating these strategies significantly intensifies the upstream workflow and improves scalability, ultimately making mAb production more efficient. The synergy created by using a high-performance medium and an optimized feed strategy in intensified bioreactor processes further elevates productivity and operational efficiency. This holistic approach enables each technology to complement the others, resulting in a seamless and highly efficient workflow for intensified mAb production.

This white paper illustrates how combining intensification strategies can double upstream product yield from the average industry standard of ~4 g/L to 8.4 g/L; accelerate manufacturing speed; reduce labor and consumable costs; and enhance the sustainability of the entire production process. All economic modeling data presented herein were generated using BioSolve Process™ software. The modeling assumptions are detailed in Appendix I.

Enhancing mAb production with high-density cell banks: speed and efficiency gains

Using a HDCB is an effective way to accelerate upstream manufacturing. Derived from well-characterized master cell banks, HDCBs are reliable, consistent, and contaminant-free cell sources for efficient mAb production. HDCBs contain high concentrations of viable cells, which allow rapid scale-up and faster expansion to desired production levels. This shortens the cell growth and seed train timeline, ultimately speeding up the overall manufacturing process. HDCBs can also help mAb manufacturers make optimal use of resources like media, nutrients, and bioreactor space to reduce waste and operational costs, thereby making the process more economical.

Generating a HDCB begins with establishing a master cell bank (MCB), which is then scaled up through passaging in shake flasks. In the HDCB generation workflow shown in Figure 1, the MCB culture is transferred to the 50 L Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), and perfusion culture is employed to boost cell density. Perfusion culture enables the cell density to exceed 45 × 10^6 cells/mL while preserving the exponential growth phase needed for cell banking and maintaining cell quality and viability for subsequent steps. An automated filling system accelerates the filling and freezing steps, which is critical for preserving cell viability. The workflow reduces the number of open steps, which reduces the risk of costly deviations and process failures associated with manual operations. HDCBs produced using the DynaDrive S.U.B. are also robust and exhibit performance that is comparable to that of standard cell lines.

Using a HDCB can significantly improve the seed train process. In the example outlined in Table 1, use of a HDCB reduced process duration from 18 days to 9 days, the number of required full-time employee (FTE) operators from 14 to 5, and the production area required from 957 m^2 to 721 m^2 (25%).

Early seed train operations are resource-intensive, as they require skilled operators and process control quality monitoring. High-density cell banking can limit early seed train steps, thereby reducing labor costs and streamlining upstream processes with automatable closed bioreactors.

Incorporating automated steps also saves time and reduces consumable use as well as the need for capital investments in bioreactors, incubators, and monitoring equipment. For example, removing a 70 m^2 host cell laboratory from a greenfield project could save over $750,000 in capital costs (see Appendix I).

Table 1. Impact of using a high-density cell bank on the seed train process.

	Standard process	HDCB process	Reduction
Process area (m^2)	957	721	25%
Number of FTE operators	14	5	64%
Seed train duration (days)	18	9	50%

Key takeaways:

- High-density cell banks reduce the amount of time needed for cell growth and proliferation, speeding up the seed train process and overall mAb manufacturing.
- HDCBs can help manufacturers make optimal use of resources (e.g., media, nutrients, bioreactor space), reduce waste, and bring down operating costs for faster and more cost-effective mAb manufacturing.

Boosting mAb yield: the impact of N-1 perfusion and intensified fed-batch cell culture

Perfusion culturing and intensified fed-batch culturing methods are used during the seed train cell expansion and production stages of mAb manufacturing, respectively. While both approaches aim to optimize cell growth and product yield, they have different cell retention and product removal strategies. Perfusion cultures maintain logarithmic cell growth, enabling high cell densities with fewer vessels in the seed train. Conversely, intensified fed-batch cultures are inoculated at high seeding densities to avoid the lag phase and increase viable cell density (VCD) more quickly, which helps improve the final titer within a 14-day production cycle.

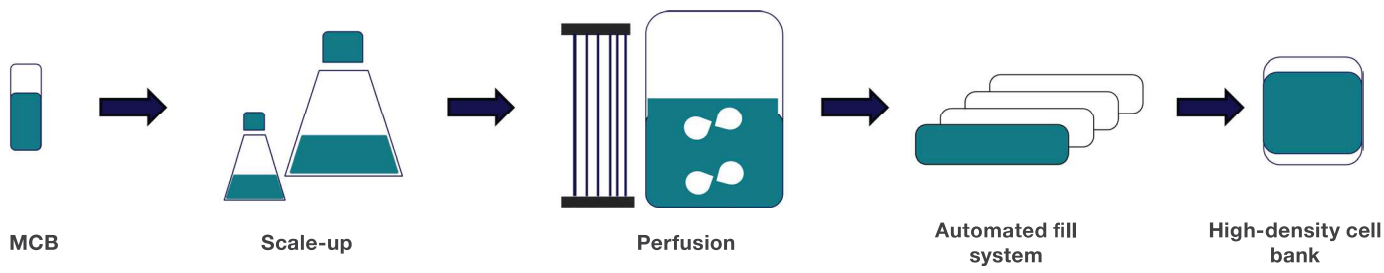

Figure 1. HDCB generation workflow.

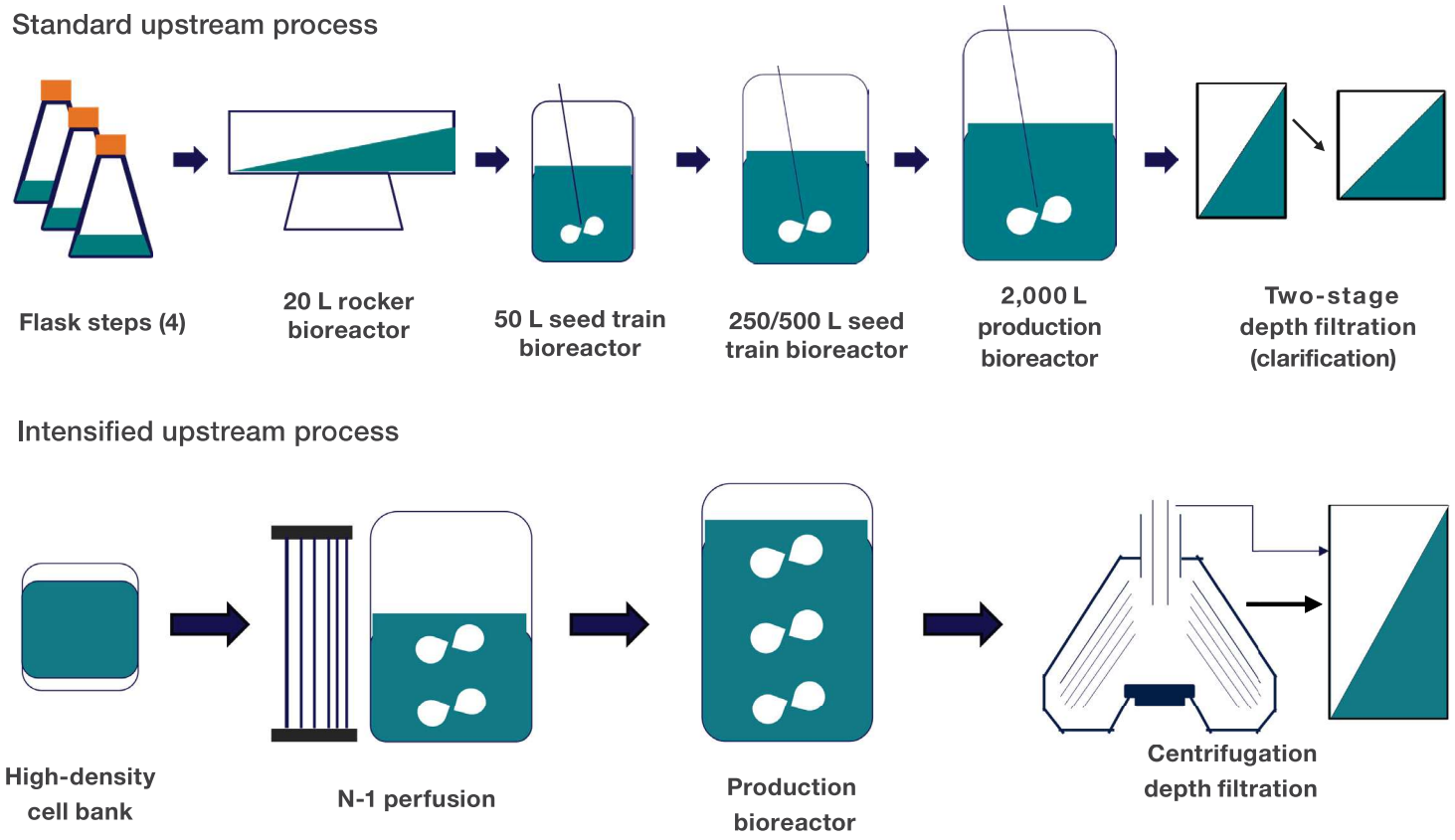

Figure 2. Comparison of standard and intensified upstream process workflows. The intensified process workflow reduces the number of steps required, thereby speeding up the process.

Using N-1 perfusion and intensified fed-batch culturing methods in a complementary fashion has an additive effect. N-1 perfusion culturing produces a healthy, high-density inoculum that can be rapidly scaled up in a production bioreactor (Figure 2). Intensified fed-batch culturing maintains high-density cultures under optimal conditions throughout the production phase, resulting in more efficient bioprocesses and higher overall mAb yields.

Implementing N-1 perfusion and intensified fed-batch culturing methods can significantly enhance bioprocess efficiency (Figure 3). In this example, it doubled the maximum VCD from 20 x 10^6 cells/mL to 40 x 10^6 cells/mL, and peak VCD was reached by day 4 instead of day 7. The product titer rose from 3.2 g/L to 4.3 g/L, a 34% increase relative to the standard process. This strategy resulted in higher cell densities and product yields within a shorter timeframe, thereby enhancing scalability and economic viability.

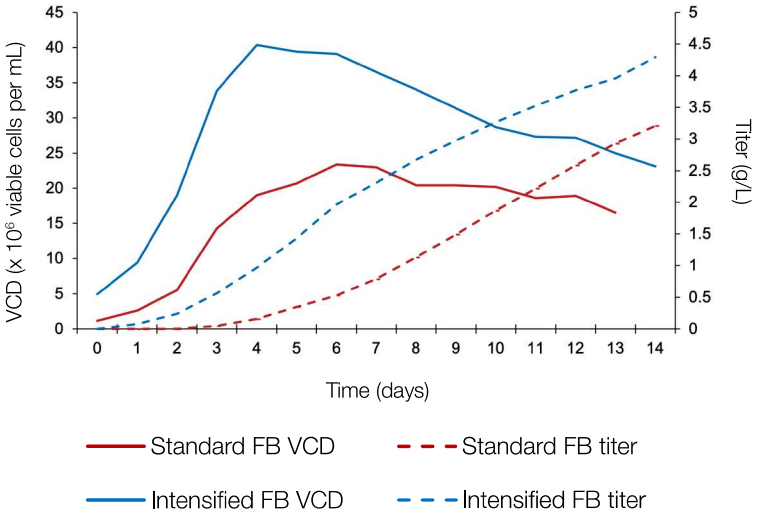

Figure 3. Impact of N-1 perfusion and intensified fed-batch culturing on mAb production yield. VCD and product titer were both higher relative to the standard fed-batch (FB) culturing process.

High turndown ratios enable optimal culture conditions to be maintained across a broad range of volumes. This reduces the time and number of single-use vessels and consumables needed for the seed train process and supports consistent product quality and yield. The 50 L DynaDrive S.U.B. has a 10:1 turndown ratio and a minimum operating volume of 5 L, so N-2 and N-1 steps can be completed in the same vessel. This can be followed by direct inoculation into the 500 L Thermo Scientific™ DynaDrive™ S.U.B., which has a turndown ratio of 20:1 and a minimum operating volume of 25 L [1,2]. DynaDrive S.U.B.s can thus help minimize downtime and boost overall efficiency by swiftly adapting to changing production demands.

In the example outlined in Table 2, pairing N-1 perfusion and intensified fed-batch culturing methods and using a HDCB led to significant improvements in mAb production. Specifically, the product titer increased from 3.2 g/L to 4.3 g/L, and cell yield rose from 461 kg per year to 623 kg per year. The process area required was reduced from 957 m^2 to 683 m^2, and FTE labor hours decreased from 204 to 163, making the manufacturing process faster and more efficient.

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

The intensified fed-batch process included: 1) a HDCB; 2) a seed train process with an N-2 recovery step and N-1 perfusion step in the 50 L DynaDrive S.U.B.; and 3) an intensified fed-batch production step with high-VCD inoculum (Table 3).

Table 2. Impact of process intensification on mAb production.

	Standard process	Intensified process	Improvement
Cell titer	3.2 g/L	4.3 g/L	34%
Annual throughput (kg/year)	460.6	622.7	34%
Cost per gram (USD)	$247	$203	18%
Process area (m^2)*	957	683	29%
FTE labor**	204	163	20%

* Process area savings: $1.6M. See Appendix I for assumptions.

** FTE labor savings: $3.8M. See Appendix I for assumptions.

Table 3. Overview of technologies used in the standard and intensified fed-batch processes.

	Standard process	Intensified process
Working cell bank	Standard vial	HDCB
Seed train process	Expansion in shake flasks; N-2 and N-1 batch in the 50 L DynaDrive S.U.B.	N-2 recovery and N-1 perfusion steps in the 50 L DynaDrive S.U.B
Production	Standard fed-batch production with standard VCD inoculum	Intensified fed-batch process with high VCD inoculum

Key takeaways:

- Pairing N-1 perfusion culture and intensified fed-batch culture has an additive effect that leads to more efficient and productive bioprocesses with higher cell densities and product yields in less time.
- The high turndown ratios of DynaDrive S.U.B.s minimize the time and number of vessels needed for the seed train process, enabling more efficient use of resources.

Improving mAb production efficiency: advantages of single-use systems

Utilizing single-use systems, which include bioreactors, centrifuges, and other components, can help reduce the upstream manufacturing footprint of fast and efficient mAb production [3]. In addition to reducing downtime and utility demands, single-use systems can substantially reduce space requirements by eliminating the need for an extensive cleaning and sterilization infrastructure [4]. Selecting compact, flexible, and easily replaceable single-use systems streamlines logistics and simplifies setup for a more efficient, scalable, and environmentally sustainable manufacturing process.

Using the Thermo Scientific™ DynaSpin™ Single-Use Centrifuge during the harvest step in high-density processes enables >90% clearance of cells [5], which significantly reduces the number of filters needed for the clarification process. When compared to conventional depth filtration, the DynaSpin centrifuge can reduce depth filter usage by 72% as well as reduce the volume of the downstream processing pool, the number of FTE hours required, and the equipment footprint [6].

The reduction in depth filter usage becomes even more significant as packed cell volume increases, so the efficiency gains from intensifying this step can be substantial. Using the DynaSpin Single-Use Centrifuge can also help reduce buffer and water consumption [7] to increase the sustainability of the manufacturing process. Using the DynaSpin Single-Use Centrifuge in place of a standard depth filtration step can significantly enhance process efficiency.

In the example outlined in Table 4, the DynaSpin centrifuge increased depth filter capacity from 60 L/m^2 to 150 L/m^2—a 2.5-fold improvement—and reduced the labor hours required by 20%. It also cut the equipment footprint by three-quarters from 17 m^2 to 4 m^2. Using a HDCB together with DynaDrive S.U.B.s and the DynaSpin Single-Use Centrifuge can reduce the manufacturing footprint by approximately 236 m^2 and enable faster and more efficient mAb production.

Table 4. Harvest with the DynaSpin Single-Use Centrifuge vs. standard depth filtration for mAb production.

	Depth filtration (standard process)	Harvest with the DynaSpin Single-Use Centrifuge	Improvement
Filter capacity (L/m^2)	60	150	▲ 150%
Pool volume (L)	550	496	▼ 10%
Water usage (L)	1,980	550	▼ 72%
Total labor hours	5	4	▼ 20%
Equipment footprint (m^2)	17	4	▼ 76%
Depth filter usage	18	5	▼ 72%
Depth filter type	Two-stage	Single-stage	–

Key takeaways:

- The DynaSpin Single-Use Centrifuge achieves >90% cell clearance and reduces the number of depth filters required for clarification by 76%.
- Using the DynaSpin Single-Use Centrifuge can also reduce the downstream processing pool volume, the number of labor hours required, and the equipment footprint.

Transforming mAb production: the impact of high-production CHO cell lines and media

Media that provide optimal nutrients and growth conditions for cell lines engineered for high-level mAb production can enhance cell viability, support higher cell densities, and help ensure stable and consistent production without compromising product quality. Using a high-production Gibco™ CHOvantage™ Cell Line with optimized medium, feed, and enhancer in an intensified upstream workflow that includes a HDCB, N-1 perfusion and intensified fed-batch culturing methods, and single-use bioreactor and centrifugation systems can increase mAb yield. The scalability of these systems can also facilitate the transition from small-scale to large-scale production for cost-effective manufacturing. In the process described in this paper, the transition from N-2 and N-1 and inoculation of the 500 L DynaDrive S.U.B. were fully automated with the Thermo Scientific™ G3Pro Bioprocess Controller and managed through Thermo Scientific™ TruBio™ Bioprocess Automation Software based on capacitance readings of viable cell volume (VCV).

Using high-production CHOvantage cells together with Gibco™ Efficient-Pro™ Medium, Gibco™ Efficient-Pro™ Feed 3, and Gibco™ Efficient-Pro™ Feed Enhancer in the intensified process resulted in significantly higher specific productivity. This approach nearly doubled the cell titer from 4.3 g/L to 8.4 g/L when compared to the traditional intensified process. However, the maximum VCD was reduced from over 40 x 10^6 cells/mL to 30 x 10^6 cells/mL (Figure 4).

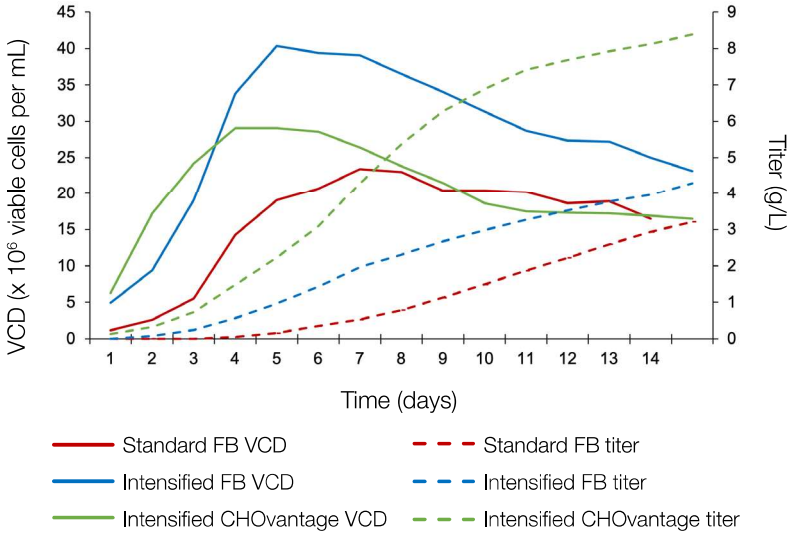

Figure 4. Including the CHOvantage Cell Line in the intensified process resulted in the greatest increase in VCD and cell titer.

The combined approach significantly improved product yield, raising the titer from 3.2 g/L to 8.4 g/L (Table 5). Considering the standard titer range of 3–5 g/L, this represents a 110% increase in manufacturing output that could translate to an annual production boost from 461 kg to 1,216 kg. Consequently, the potential profit in this scenario could increase from $24 million to $224 million (800%). As throughput increases, the manufacturing cost of goods (COG) would decrease by >50% from $247/g to $116/g (Figure 5).

Key takeaways:

- Using high-production CHOvantage cells can result in enhanced cell viability and support higher cell densities for stable, consistent production without compromising quality.
- Manufacturing yield can be increased by 110% over the current industry standard of 3–5 g/L.
- Implementing an intensified process with CHOvantage cells can potentially increase profits by 800% and provide a potential return on investment (ROI) of $192M (Table 6).

Table 5. Impact of using CHOvantage cells and optimized medium in the intensified process for mAb production.

	Standard process	Intensified process	Intensified process with CHOvantage Cell Line	Improvement over standard process
Cell titer	3.2 g/L	4.3 g/L	8.4 g/L	**163%**
Annual throughput (kg/year)	461	623	1,216	**164%**
Cost per gram (USD)	$247	$203	$116	**53%**
Estimated profit*	$24M	$60M	$224M	**800%**

* The model assumes a mAb sale price of $300 per gram. Profit is dependent on annual manufacturing output and the actual mAb sales price. See Appendix I for assumptions.

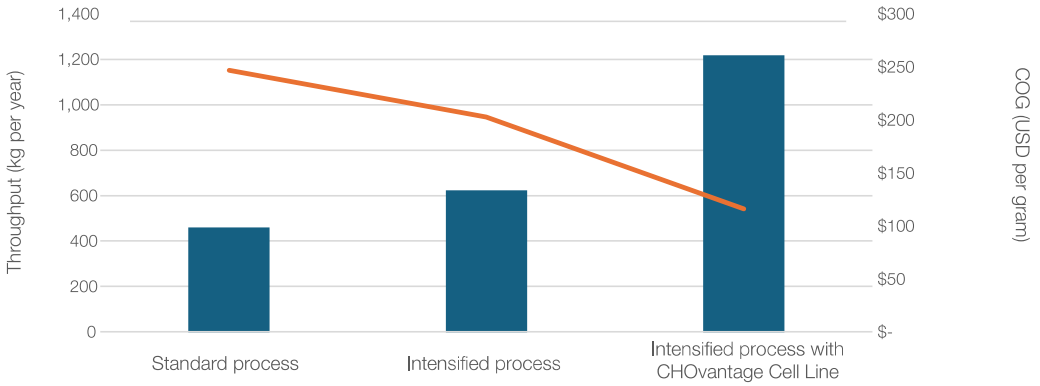

Figure 5. Throughput and manufacturing COG of an intensified process performed with CHOvantage cells. Use of the CHOvantage Cell Line in the intensified process increased throughput and reduced the COG when compared to the standard process.

Table 6. Investment versus return on investment for adopting the intensified process plus CHOvantage Cell Line.

	Standard process	Intensified process	Intensified process plus CHOvantage Cell Line	Investment or savings
Capital expense for new facility*	$55M	$57M	$61M	**$6M**
Operational expense for batch	$1.06M	$1.15M	$1.28M	**$216K**
Facility expense/year	$116M	$126M	$141M	**$25M**
			Total investment	**$31M**
Facility FTE**	204	162	200	**$600K**
Annual throughput†	461 kg	623 kg	1,202 kg	**$222M**
			Total savings/profit	**$223M**
			Return on investment‡	**$192M**

* Assumption: new facility size for 6 x 2,000 L.
** Assumption: average FTE salary of $150K/yr.
† Assumption: average mAb sale price of $300/g. Profit is dependent upon annual manufacturing output and actual mAb sale price (see Appendix I for assumptions).
‡ ROI is calculated by subtracting the total investment from the total savings/profit.

Conclusion

Integrating a high-density cell bank, N-1 perfusion culture, intensified fed-batch culture, and single-use bioreactor and centrifugation systems in an intensified process can significantly enhance mAb production. In addition to improving product yield, this combination of strategies also reduces the footprint and increases the efficient resource management in mAb manufacturing. These advancements collectively streamline the production process, minimize resource consumption, and maintain consistent product quality, positioning them as pivotal drivers of future advances in mAb manufacturing.

Including a high-producing CHOvantage Cell Line and an optimized medium and feed system in our intensified process resulted in the greatest increase in product yield and greatly improved mAb production efficiency. This combination raised the product titer from 4.3 g/L to 8.4 g/L, which translated to an estimated 800% boost in profit and a 50% reduction in manufacturing costs in our model.

Although the adoption of this intensified process plus the high-producing cell line and optimized media require an initial capital investment of $31M, the return on investment (ROI) for doing so is $192M (Table 6). This significant ROI illustrates the benefit of adopting an optimized strategy for upstream manufacturing workflows. These innovations represent a holistic approach for intensifying mAb production, with each technology complementing the others. This seamless and highly efficient workflow can accelerate time-to-market for life-saving treatments, and it can reshape expectations for the future of mAb manufacturing.

Appendix I

Assumptions for economic modeling with BioSolve Process software

- List price (no discount applied)
- Software used for labor and facility analysis
- 12-month campaign length, 80% facility efficiency
- 6 x 2,000 L bioreactor production process
- 18 batches per year or 80% utilization
- Standard process with seven seed train steps
- ~70% process yield
- 8% cost of capital
- Sustainability modeling assumes facility is in Massachusetts, USA
- mAb sale price of $300 per gram
- Process area cost based on a Class D cleanroom at a price of $5,900 m^2
- FTE labor cost based on an average FTE annual compensation package of $93,000, unless otherwise noted

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Disclaimer

The data and case studies presented in this document are provided for informational purposes only and are intended to illustrate potential process and economic outcomes associated with the use of bioprocess technologies from Thermo Fisher Scientific. The analyses include examples of standard fed-batch and intensified upstream processing, as well as traditional and intensified downstream protein purification workflows, modeled using BioSolve Process software by Biopharm Services.

All modeling and performance data are representative of actual running scenarios designed to demonstrate the possible value of process intensification strategies. These results are not intended to predict or guarantee actual process performance or financial outcomes. Process results, including yield, productivity, and cost efficiency, will vary based on multiple factors such as equipment configuration, process parameters, scale, and facility design.

The data illustrate how the combination of high-performing media, optimized feed strategies, and intensified bioreactor operation can enhance productivity and operational efficiency in upstream cell culture, while integration with intensified downstream purification approaches can further improve throughput, reduce consumable and labor costs, and enhance process sustainability.

The example comparisons between standard and intensified workflows—such as potential yield improvements from an industry average of 4 g/L to approximately 8.4 g/L—are provided to demonstrate the potential benefits of integrated intensification strategies.

Thermo Fisher Scientific does not guarantee or warrant specific outcomes for any customer implementation. Each process must be evaluated and optimized based on individual operational and technical requirements.

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