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Application Note

Optimizing Feed Strategies for Intensified Fed-Batch with Cellvento® ModiFeed Prime Feed

Intensified fed-batch processes present unique challenges and opportunities in biopharmaceutical production. For example, not all commercially available Chinese Hamster Ovary (CHO) cell culture media and feeds suitable for conventional fed-batch applications can adequately meet the heightened nutrient demands of intensified approaches. In addition, the feeding strategies required for intensified fed-batch processes differ significantly from those used in traditional fed-batch applications.

This study investigates the impact of initiating feeding on day 0, rather than day 3 as in conventional fed-batch processes, to support high inoculation densities. It also evaluates different feeding schedules to optimize performance.

The results demonstrate that equivalent productivity can be achieved in just 9 days using intensified fed-batch techniques, compared to 14 days with traditional fed-batch. Among the multiple feeding strategies tested, two preferred feeding approaches were identified, presenting an opportunity to streamline future process evaluation. By focusing on these two strategies, researchers can efficiently determine the optimal feeding regimen for their specific cell line, significantly reducing the time and resources required for process development.

Promise of Intensified Fed-Batch

Process intensification plays a crucial role in optimizing biopharmaceutical production by enhancing efficiency, productivity, and cost-effectiveness. One significant approach to process intensification is the implementation of intensified fed-batch strategies. Compared to perfusion processes, intensified fed-batch offers several advantages that make it a promising alternative, especially at the production stage.

Intensified fed-batch is defined as a fed-batch process in which the inoculation density of the production (or N stage) bioreactor is increased from the traditional approximately 0.5 × 10^6 viable cells/mL to 2–10 × 10^6 viable cells/mL. This increased inoculation density

is often facilitated by the intensification of the N-1 expansion stage through perfusion technology. Intensifying the N stage allows for increased manufacturing output, either by achieving the same productivity in a shorter production run or by attaining higher productivity in a production run of the same length. Both scenarios enable processes that increase product yield, supporting reduced COGS and better capacity utilization, thereby advancing next generation bioprocessing goals (**Figure 1**).

Although intensified fed-batch is among the most promising elements of upstream process intensification due to its relative ease of implementation, it still presents challenges compared to traditional fed-batch methods. While various options exist for scaling up, upstream process intensification effectively addresses the challenges of rapidly generating sufficient biomass to inoculate an N stage bioreactor at 2–10 × 10^6 viable cells/mL. Additionally, the fed-batch media Cellvento® 4CHO COMP and EX-CELL® Advanced CHO, combined with the Cellvento® ModiFeed Prime Feed, successfully sustain the high cell densities required to fully leverage the benefits of intensified fed-batch processes. **Table 1** shows an overview of the media and feed materials used in this study.

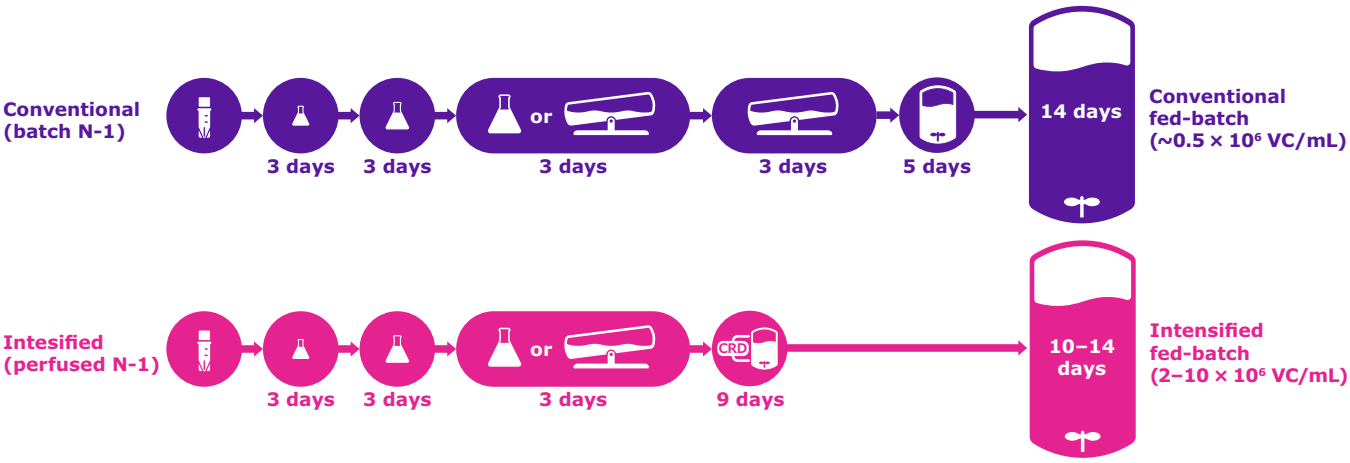

Figure 1.

Comparison of the conventional fed-batch process and the intensified fed-batch process, highlighting key differences in seeding density and duration.

Table 1.

Fed-batch media and feed evaluated in intensified batch and fed-batch processes.

		Concentration [g/L]	Osmolality range [mOsm/kg]	pH range	Catalog number and format
Medium	**Cellvento® 4CHO COMP**	23.7	280–340	6.7–7.3	103795 Compacted Dry Powder
Medium	**EX-CELL® Advanced CHO Fed-batch**	22.1	280–320	7.1–7.3	24366C Dry Powder 14366C Liquid
Feed	**Cellvento® ModiFeed Prime COMP**	131.6	1175–1315	6.4–7.0	104132 Compacted Dry Powder

Impact of Day 0 Feeding on Media Formulations

Increased inoculation density may require a higher supply of nutrients at the initiation of an intensified fed-batch process and during the early growth phase. Traditional fed-batch media are designed to support batch culture and the initial nutrient demands of conventional fed-batch processes. In this study, we utilize the media Cellvento® 4CHO COMP and EX-CELL® Advanced CHO Fed-batch Medium, both of which are chemically defined cell culture media developed for the growth of CHO cells and the expression of monoclonal antibodies (mAbs) and recombinant proteins in batch and fed-batch processes.

To accommodate increased inoculation density, standard fed-batch medium can be augmented by adding feed on day 0, thus increasing the amount of total nutrients. In addition, the standard fed-batch medium may require glucose supplementation depending on the intended control strategy. In this study, we utilized Cellvento® ModiFeed Prime Feed, a highly concentrated, one-part, pH neutral feed designed to replenish depleted nutrients necessary for cellular function and to extend the production phase of fed-batch cultures. Cellvento® ModiFeed Prime Feed was used both as a day 0 feed to enhance the media and as a feed throughout the intensified fed-batch process to support culture longevity and productivity. Supplementation with glucose is particularly important when using Cellvento® ModiFeed Prime Feed, as it does not contain any glucose. For this reason, glucose was supplemented at the very beginning and monitored closely throughout the process.

Additions of 3.5% or 4.5% of Cellvento® ModiFeed Prime Feed were selected for evaluation, aligning with feeding percentages used in traditional fed-batch processes. Glucose was increased to 10 g/L to ensure a sufficient supply during the early culture phase. With either percentage, the osmolality of both Cellvento® 4CHO COMP and EX-CELL® Advanced CHO Fed-batch media was maintained below the target of 370 mOsm/kg, ensuring a physiologically relevant osmolality at the initiation of the intensified process (**Table 2**).

Table 2.

Impact of day 0 feeding of Cellvento® 4CHO COMP Medium and EX-CELL® Advanced CHO Fed-batch Medium with Cellvento® ModiFeed Prime Feed on initial osmolality.
Media glucose was raised to 10 g/L total and a percentage (3.5% or 4.5%) of hydrated Cellvento® ModiFeed Prime Feed was added to hydrated Cellvento® 4CHO COMP and EX-CELL® Advanced CHO Fed-batch media.

	Cellvento® 4CHO COMP Medium		EX-CELL® Advanced CHO Fed-batch Medium	
	Osmolality [mOsm/kg]	**Media Final [g/L]**	**Osmolality [mOsm/kg]**	**Media Final [g/L]**
Unmodified	312	23.7	308	22.1
Glucose Raised to 10 g/L Total	329	27.7	321	25.1
Glucose Raised to 10 g/L Total + 3.5% Feed Added	359	32.3	349	29.7
Glucose Raised to 10 g/L Total + 4.5% Feed Added	367	33.6	360	31.0

Importance of Day 0 Feeding on Intensified Batch Culture

Two mAb-producing CHOZN® clones were tested in intensified batch culture to assess the impact of increased nutrients alongside inoculation densities of approximately 3.5 and 7 × 10^6 viable cells/mL. Bioreactor spin tubes, with an initial working volume of 25 mL, were incubated at 37 °C, 5% CO_2, 80% humidity and 320 rpm. Glucose levels were monitored and supplemented as needed.

The increased nutrients provided by Cellvento® ModiFeed Prime Feed were essential for sustaining the intensified culture during the first 2–3 days, depending on the clone and seeding density.

Although no significant difference in performance between feeding 3.5% and 4.5% Cellvento® ModiFeed Prime Feed on day 0 was observed, it is evident that day 0 feeding is crucial for the longevity of this process (Figure 2).

Feeding Schedule Evaluation

Building on the initial intensified batch experiment, day 0 feeding was used during feed schedule determination for intensified fed-batch processes. Due to the similar performance observed between 3.5% and 4.5% feeding on day 0, and to simplify the process, the feeding percentage on day 0 was selected to match later feeding during the culture. Feeding was initiated on day 1 or 2 for the 4.5% regimen and on day 2 or 3 for the 3.5% regimen. Regardless of the feed initiation day, cultures were fed daily for a total of 5 additional times (**Table 3**). The timing of feed administration and the total number of feeds were guided by a previous intensified fed-batch experiment (data not shown) and aimed to identify the optimal approach for each clone to maximize productivity.

To further assess the robustness of the approach, a third clone, CHOK1 GS, producing another mAb, was introduced. Bioreactor spin tubes were again employed with intensified cultures seeded at 7 × 10^6 viable cells/mL. Control schedules were based on optimal feeding determined for each clone using Cellvento® ModiFeed Prime Feed in traditional fed-batch (**Table 3**).

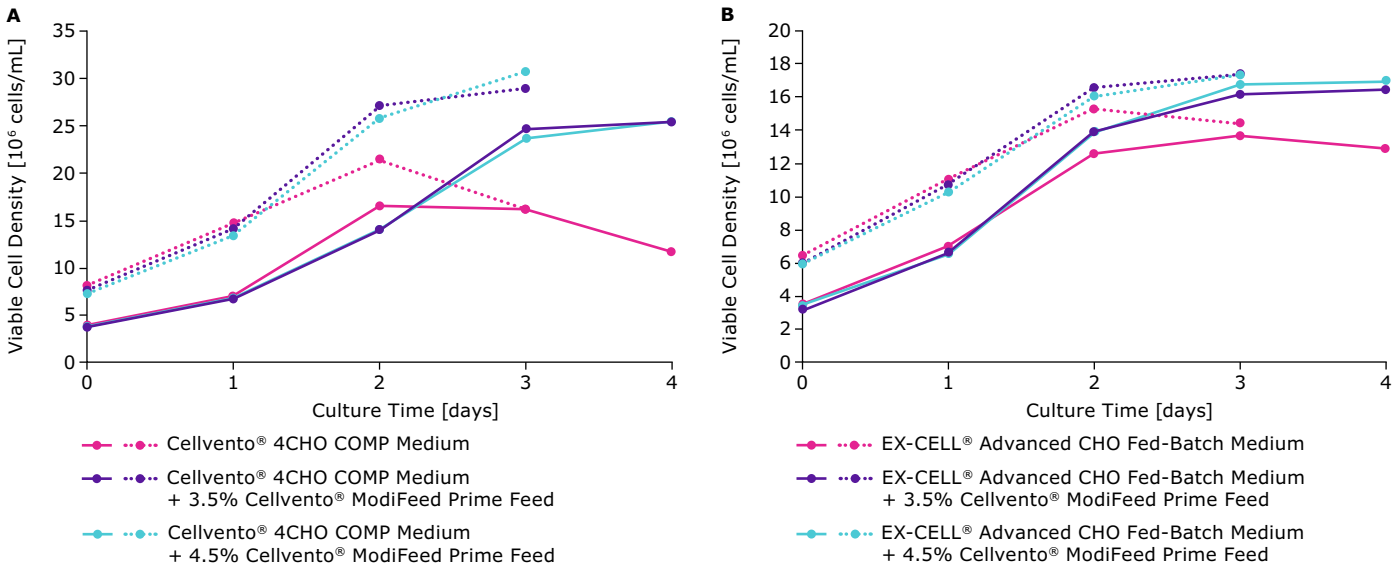

Figure 2.

Impact of day 0 feeding with different amounts of Cellvento® ModiFeed Prime Feed (3.5% or 4.5%) on viable cell density during a 3- or 4-day batch process seeded at 3–4 × 10^6 viable cells/mL (solid lines) and 6–8 × 10^6 viable cells/mL (dashed lines): (**A**) CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium and (**B**) CHOZN® mAb2 clone in EX-CELL® Advanced CHO Fed-batch Medium.

Table 3.

Fed-batch feeding schedules showing the quantities of Cellvento® ModiFeed Prime Feed per day.

Day	0	1	2	3	4	5	6	7	8	9	10	11
CHOZN® mAb1 Control				4.5%		4.5%		4.5%		4.5%		4.5%
CHOZN® mAb2 & CHOK1 GS mAb3 Control				3.5%		3.5%		3.5%		3.5%		3.5%
Schedule 1	4.5%	4.5%	4.5%	4.5%	4.5%	4.5%						
Schedule 2	4.5%		4.5%	4.5%	4.5%	4.5%	4.5%					
Schedule 3	3.5%		3.5%	3.5%	3.5%	3.5%	3.5%					
Schedule 4	3.5%			3.5%	3.5%	3.5%	3.5%	3.5%				

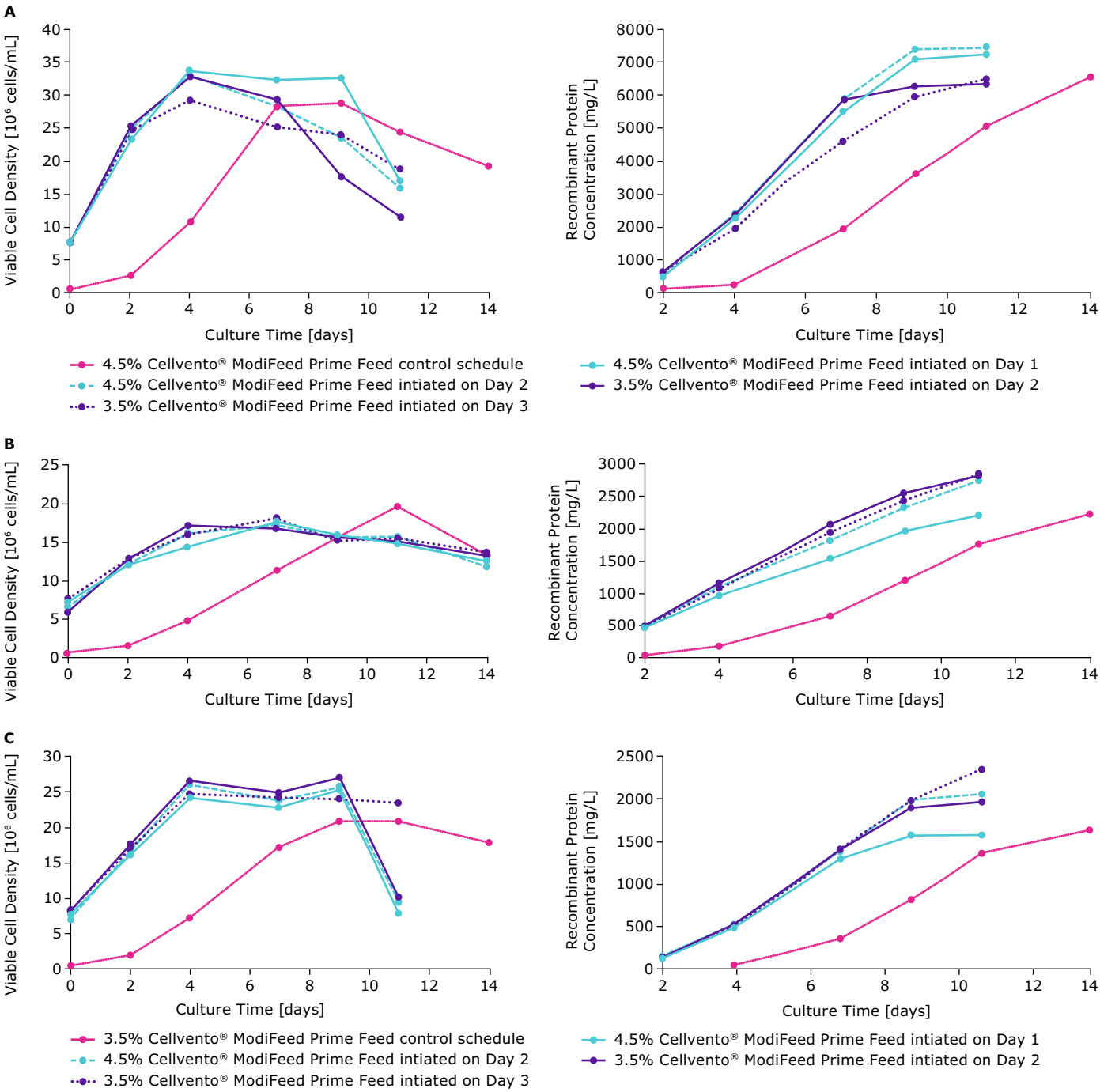

Figure 3.

Impact of feeding 3.5% or 4.5% Cellvento® ModiFeed Prime Feed on viable cell density and productivity during a 14-day intensified fed-batch process seeded at 4 × 10^5 viable cells/mL (control pink line) or 7 × 10^6 viable cells/mL: (**A**) CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium; (**B**) CHOZN® mAb2 clone in EX-CELL® Advanced CHO Fed-batch Medium; (**B**) CHOK1 GS mAb3 clone in Cellvento® 4CHO COMP Medium.

For CHOZN® mAb1 clone initiating feeding of 3.5% on culture day 3 resulted in decreased viable cell density (**Figure 3A**). This clone responded best to the increased feeding amount of 4.5%, with the highest productivity observed when feeding was initiated on culture day 2 (**Figure 3A**). There was minimal difference in the growth profile of both CHOZN® mAb2 and CHOK1 GS mAb3 clones (**Figure 3B & 3C**). Only the 4.5% feed initiated on culture day 1 led to a reduction in productivity for CHOZN® mAb2 clone (**Figure 3B**), while CHOK1 GS mAb3 clone showed a clear preference for the 3.5% feed initiated on culture day 3 (**Figure 3C**), sustaining high viable cell density through day 11.

For all these cell lines, productivity equivalent to the control condition was achieved in the preferred intensified fed-batch conditions before day 9, which is five days earlier than in traditional fed-batch processes. Extending the intensified conditions to just day 11 resulted in a 14–44% increase in productivity compared to the control.

Controlled Environment

To confirm the performance in intensified fed-batch processes, the preferred feeding strategies were evaluated in a controlled environment. Based on positive results from the bioreactor spin tube experiments, a day 0 feed was used for the intensified inoculations. The impact of reducing the number of total feeds from 6 to 5 (**Table 4**) was assessed to determine if equivalent productivity could be maintained while lowering the higher osmolality, lactate and ammonia levels typically observed in intensified fed-batch (data not shown). The use of a microbioreactor that allows process control of pH and dissolved oxygen is beneficial, as these may also influence cell culture performance.

Due to variability in the industry in what inoculation density constitutes 'intensified,' two different seeding densities were examined during bioreactor evaluation. The demands of cultures seeded at 4×10^6 viable cells/mL may differ significantly from those seeded at 9×10^6 viable cells/mL. By using these two inoculation densities, we aimed to determine whether a preferred feeding schedule established at 7×10^6 viable cells/mL in bioreactor spin tubes could be applicable to other intensified inoculation densities without requiring substantial additional process development.

Table 4.

Fed-batch feeding schedules showing the quantities of Cellvento® ModiFeed Prime Feed per day.

Day	0	1	2	3	4	5	6	7	8	9	10	11
Schedule 1	4.5%		4.5%	4.5%	4.5%	4.5%	4.5%					
Schedule 2	4.5%		4.5%	4.5%	4.5%	4.5%						
Schedule 3	3.5%			3.5%	3.5%	3.5%	3.5%	3.5%				
Schedule 4	3.5%			3.5%	3.5%	3.5%	3.5%					

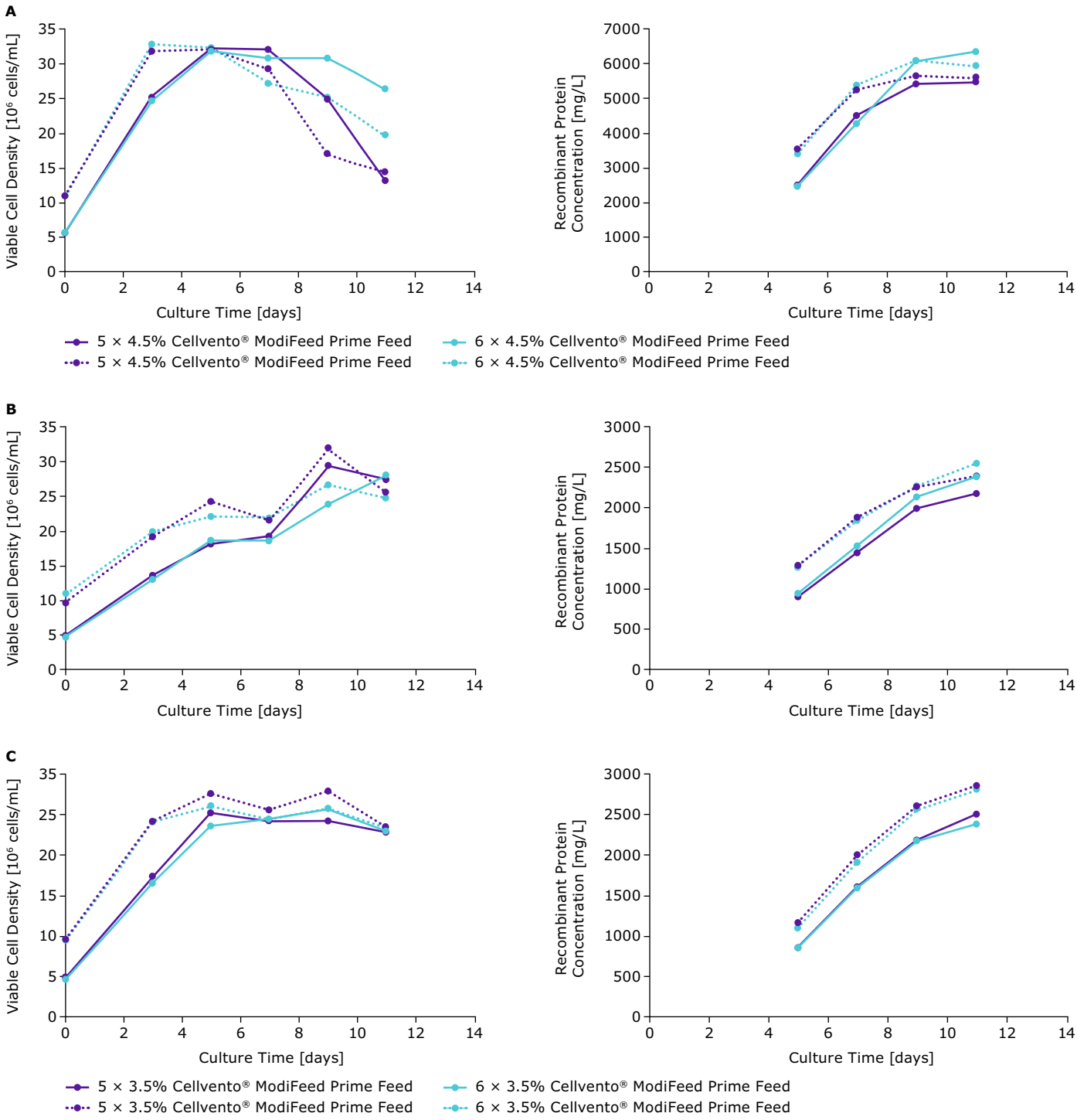

Figure 4.

Impact of feeding 3.5% or 4.5% Cellvento® ModiFeed Prime Feed on viable cell density and productivity during a 11-day intensified fed-batch process seeded at 4 × 10^6 viable cells/mL (solid lines) or 9 × 10^6 viable cells/mL (dashed lines): (**A**) CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium; (**B**) CHOZN® mAb2 clone in EX-CELL® Advanced CHO Fed-batch Medium; (**C**) CHOK1 GS mAb3 clone in Cellvento® 4CHO COMP Medium.

For CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium, the higher number of total feeds (6 instead of 5) maintained higher viable cell densities for a longer period of time (**Figure 4A**). This was true for both inoculation densities. The sustained viable cell density translated into increased recombinant protein production of over 10% for CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium, regardless whether it was seeded at 4 × 10^6 or 9 × 10^6 viable cells/mL (**Figure 4A**). In contrast, using the 3.5% feeding regimen there was no positive impact of 6 feeds on viable cell density and productivity for both CHOZN® mAb2 clone in EX-CELL® Advanced CHO Fed-batch Medium and CHOK1 GS mAb3 clone in Cellvento® 4CHO COMP Medium. Seeding the culture at the inoculation density of 9 × 10^6 viable cells/mL showed potential for increased productivity, particularly for CHOK1 GS mAb3 clone in Cellvento® 4CHO COMP Medium (**Figure 4C**). The preferred feeding schedule was consistent across the two inoculation densities.

Table 5.

Impact of feeding 3.5% or 4.5% Cellvento® ModiFeed Prime Feed on osmolality, lactate and ammonia during an 11-day intensified fed-batch process seeded at 4 × 10^6 viable cells/mL or 9 × 10^6 viable cells/mL. CHOZN® mAb1 in Cellvento® 4CHO COMP.

		Day 11		
Total Number and Percentage of Feed Adds	**Inoculation Density [viable cells/mL]**	**Osmolality [mOsm/kg]**	**Lactate [g/L]**	**Ammonia [mM]**
5 × 4.5% Cellvento® ModiFeed Prime	4 × 10^6	549	4.2	2.1
6 × 4.5% Cellvento® ModiFeed Prime		569	4.0	3.9
5 × 4.5% Cellvento® ModiFeed Prime	9 × 10^6	541	3.6	2.3
6 × 4.5% Cellvento® ModiFeed Prime		581	3.5	3.7

Reducing the total feed resulted in slight positive impacts on final osmolality and ammonia levels, but had minor negative effects on final lactate levels for CHOZN® mAb1 in Cellvento® 4CHO COMP Medium. Overall, the controlled environment yielded a high productivity in a short period of time by utilizing two different intensified inoculation densities.

Given the observed potential for increased productivity with increased feeding in CHOZN® mAb1 clone and the relatively minor impact on metabolites and osmolality, a total of 6 feeds is recommended.

Improvement From Intensified Fed-Batch

The identified preferred intensified fed-batch process using EX-CELL® Advanced CHO Fed-batch Medium or Cellvento® 4CHO COMP Medium, together with Cellvento® ModiFeed Prime Feed, results in higher productivity in a shorter duration (see **Figure 5**). This shortened timeline, in turn, allows for better facility utilization, enabling a greater number of production runs over an equivalent period. More batches per year from the same facility ultimately lead to a higher yield of the final product.

As a result, this intensified fed-batch process empowers biopharmaceutical manufacturers to improve operational efficiency and accelerate mAb production.

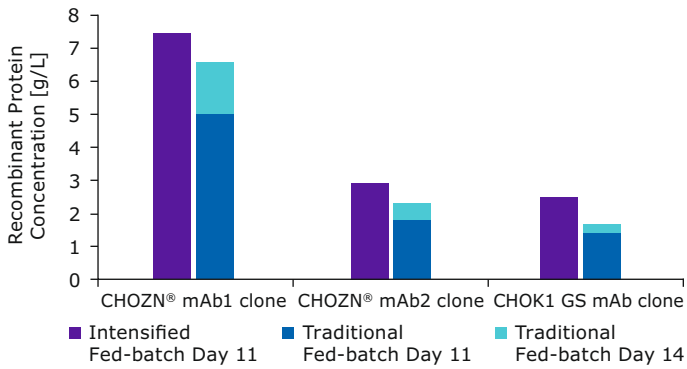

Figure 5.

Productivity during a 14-day traditional fed-batch (day 11 productivity in blue and the additional gains out to day 14 in light blue) compared to an 11-day intensified fed-batch (purple) process for CHOZN® mAb1 clone in Cellvento® 4CHO COMP Medium, CHOZN® mAb2 clone in EX-CELL® Advanced CHO Fed-batch Medium or CHOK1 GS mAb3 clone in Cellvento® 4CHO COMP Medium using the preferred Cellvento® ModiFeed Prime Feed schedule for each clone.

Future Process Development

Based on the presented results, we propose two feeding schedules for evaluating of Cellvento® ModiFeed Prime Feed in intensified fed-batch processes: **Testing these two feed schedules (Table 6) will jump-start intensified fed-batch process development**.

Given the diversity of clones, one of these two schedules will serve as a good starting point for identifying a top-performing process across a range of potential intensified inoculation densities (2–10 × 10^6 viable cells/mL) with greater efficiency.

Table 6.

Recommended feeding schedules for evaluation of Cellvento® ModiFeed Prime Feed in intensified fed-batch for diverse cell lines.

Day	0	1	2	3	4	5	6	7
Schedule 1	4.5%		4.5%	4.5%	4.5%	4.5%	4.5%	
Schedule 2	3.5%			3.5%	3.5%	3.5%	3.5%	3.5%

Conclusion

The approach to intensified fed-batch represents a significant advancement over traditional fed-batch methods. Using Cellvento® 4CHO COMP Medium or EX-CELL® Advanced CHO Fed-batch Medium in intensified fed-batch operations benefits from day 0 feeding to support the increased inoculation density and subsequent demands of higher cell densities early in the production run. The study highlights the critical role of day 0 feeding both in intensified batch and fed-batch processes, demonstrating that when combined with a more aggressive feeding strategy, it results in higher productivity.

Through testing of various feed timings and percentages, two optimal feeding schedules have been identified. The high level of performance from these two feeding schedules was maintained in a controlled environment. Consequently, these two feeding strategies represent ideal initial schedules for evaluating Cellvento® ModiFeed Prime Feed in intensified fed-batch production.

The insights gained from this study lay a robust foundation for manufacturers to streamline their process development, achieve higher productivity levels more efficiently, and accelerate their journey to market. By adopting these strategies, manufacturers can future-proof their mAb production processes, ensuring they remain competitive in an evolving market landscape.

References

1. Barna J, Horry H, Rank D. A Cost Analysis and Evaluation of Perfused Seed Train Scenarios Through Process Modeling. White paper. 2020.

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Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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