Healsun Biopharm

ONE-STOP BIOPHARMA CDMO

Comparative Analysis of N-1 Perfusion Strategies for Enhanced Protein Expression and Cost Efficiency in Biopharmaceutical Manufacturing

Background: Biopharmaceutical manufacturing relies on efficient cell culture strategies to achieve high yields of therapeutic proteins. Traditionally, conventional fed-batch culture methods have been widely used, but they come with challenges such as seed train scale-up requirements and long production durations. N-1 perfusion culture has emerged as an alternative strategy offering distinct approaches to address these challenges.

N-1 perfusion culture involves culturing cells at high densities (N-1 stage) by perfusion before transitioning to the production phase. This approach allows for the optimization of cell health and productivity before entering the main production phase. Within N-1 perfusion culture, there are two notable strategies:

1. High Dilution During Production: In this strategy, cells are diluted during the production phase to match the cell density observed in conventional fed-batch processes. This approach aims to achieve comparable production titers (g/L) while reducing the need for extensive seed train scale-up, thereby streamlining the overall manufacturing process.

2. High Cell Density During Production: Conversely, this strategy focuses on maintaining high cell density during the production phase. By keeping cells at higher concentrations, the production titer per unit volume can be increased significantly. This not only enhances productivity but also reduces the overall production duration, leading to improved cost efficiency.

Objective: This study aims to provide a comprehensive comparison of the two N-1 perfusion strategies: high dilution during production and high cell density during production. The comparison will be based on key performance metrics such as specific productivity, production titer, production duration, and overall process costs. This poster presentation will present a detailed comparative analysis of the two N-1 perfusion strategies, shedding light on their respective strengths and limitations. The findings from this study will contribute valuable insights to optimize N-1 perfusion culture strategies for improved bioprocess performance and economic viability in biopharmaceutical manufacturing.

Methods: The comparative analysis will involve conducting bioreactor experiments to evaluate the performance of each N-1 perfusion strategy. Key parameters such as cell viability, specific productivity, product titer, and production duration will be monitored and analyzed. Cost analysis will also be conducted to assess the economic implications of each strategy.

Conventional 14 day-Fedbatch

- Cell thaw 50mL
- N-2: 80mL
- N-1: 300mL
- 3L (Fedbatch: 14day)

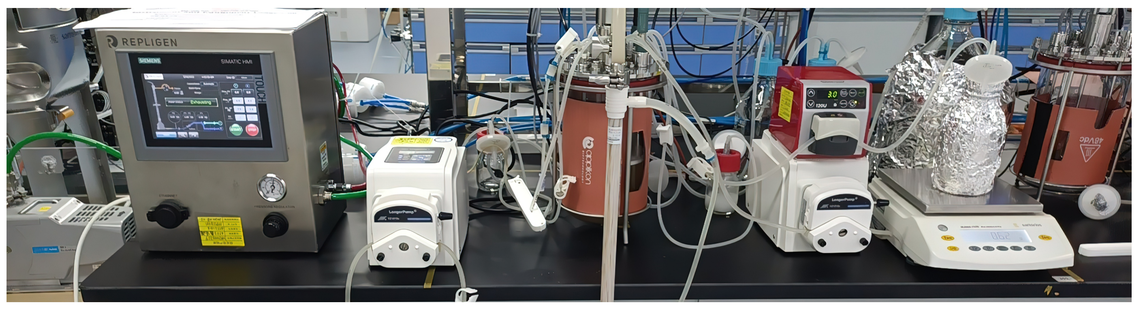
High Dilution N-1 Perfusion

- Cell thaw 50mL
- N-1: 30ml (Perfusion)
- 3L (Fedbatch: 14day)

High Seed N-1 Perfusion

- Cell thaw 50mL
- N-3: 80mL
- N-2: 300mL
- N-1: 1.5L (Perfusion)
- 3L (Fedbatch: 9-14day)

Equipment: Applikon 3L with my-Control controller, ATF2 (Repligen)
Product A: Symmetric Bispecific (172KD) ; **Product B:** Symmetric Bispecific (150KD)
Cell Type: CHO-K1 (ATCC)
Culture Medium: CD-CHO for seed train (Commercial Brand) ,HSM16 for N-1 perfusion and production scale (from Healsun)



Results:

Product A	N Seed Density	Dilution Factor	Production Culture Duration (Day)	Titer (g/L)	Cell Viability During Harvest
Conventional Production	0.6-1.0E+06	7-8	14	6.10	93.40%
N-1 High Dilution Production	0.6-1.0E+06	46	14	6.11	91.00%
N-1 High Seed Production	3.60E+07	2	9	8.71	81.80%

Product B	N Seed Density	Dilution Factor	Production Culture Duration (Day)	Titer (g/L)	Cell Viability During Harvest
Conventional Production	0.6-1.0E+07	7-8	14	5.43	94.00%
N-1 High Dilution Production	0.6-1.0E+07	80	14	5.61	93.30%
N-1 High Seed Production	6.20E+07	2	9	9.06	85.80%

Cost Estimation Analysis for 2000L Scale:

High dilution fedbatch	High seed fedbatch	Traditional fedbatch
Cell thaw 50mL	Cell thaw 50mL	Cell thaw 50mL
N-3 300mL	N-5 300mL	N-5 300mL
N-2 2L	N-4 2L	N-4 2L
N-1 20L(Perfusion)	N-3 10L	N-3 12L
2000L(fedbatch,titer:5-7g/L) Time: 14day	N-2 40L	N-2 70L
	N-1 250L	
Save equipment costs	N-11000L(Perfusion) &2000L(fedbatch, titer:8-12g/L) time:9-14day	2000L(fedbatch, titer:5-7g/L) time:14day
	Shorten cultivation time	
	Enhance production	

Process Map for 2000L scale

- High Dilution N-1 Perfusion: Shaker → 20L Bioreactor+ATF → 2000L Bioreactor
- High Seed N-1 Perfusion: Shaker → 20L Bioreactor → 250L Bioreactor → 2000L Bioreactor+ATF
- Traditional Fedbatch: Shaker → 10L Bioreactor → 50L Bioreactor → 250L Bioreactor → 2000L Bioreactor

Bioreactor trains for 2000L scale

1. High Dilution N-1 Perfusion vs. Conventional 14 day-Fedbatch
Take 2000L scale as an example for product A culture medium cost calculation

Materials	Item	Traditional fedbatch Cost of a batch (2,000L)	High dilution fedbatch Cost of a batch (2,000L)
Seed culture medium (commercial)	Unit Price (USD/L)	28	28
	Quantity (L)	80	2
	Cost (USD)	2,240	56
Basal medium (N-1 stage)	Unit Price (USD/L)	10	10
	Quantity (L)	350	130
	Cost (USD)	3,500	1,300
Basal medium (FB stage)	Unit Price (USD/L)	10	10
	Quantity (L)	1,400	1,600
	Cost (USD)	14,000	16,000
Feeding medium 1	Unit Price (USD/L)	97	97
	Quantity (L)	400	400
	Cost (USD)	38,800	38,800
Feeding medium 2	Unit Price (USD/L)	70	70
	Quantity (L)	40	40
	Cost (USD)	2,800	2,800

Item	Traditional fedbatch	High dilution perfusion
Total Price per Batch (USD)	61,340	58,956
Expression Level (g/L)	6.10	6.11
Batch Yield (g)	12,220	12,200
Cost per Gram of Protein (USD)	5.0	4.8

Cost analysis reveals compelling economic benefits associated with High dilution N-1 perfusion culture. While material costs may not significantly differ between high dilution N-1 perfusion and traditional FB processes, **considerations of labor and equipment operation costs unveil a distinct advantage for the high dilution approach.** The lower batch cost of the high dilution process, attributed to reduced labor and equipment operation expenses, positions it as a cost-effective solution. The adoption of N-1 high dilution perfusion presents a pathway to expedited processes capable of yielding multi-gram titers, thereby significantly reducing time constraints by saving seed trains and overall costs. This accelerated production not only facilitates swift pipeline delivery but also optimizes facility utilization.

2. High Seed N-1 Perfusion vs. Conventional 14 day-Fedbatch
Take 2000 L scale as an example for product B culture medium cost calculation

Materials	Item	Traditional fedbatch Cost of a batch (2000L)	High seed fedbatch Cost of a batch (2000 L)
Seed culture medium (commercial)	Unit Price (USD/L)	28	28
	Quantity (L)	80	300
	Cost (USD)	2,240	8,400
Basal medium (N-1 stage)	Unit Price (USD/L)	10	10
	Quantity (L)	350	5,000
	Cost (USD)	3,500	50,000
Basal medium (FB stage)	Unit Price (USD/L)	10	10
	Quantity (L)	1,400	800
	Cost (USD)	14,000	8,000
Feeding medium 1	Unit Price (USD/L)	97	97
	Quantity (L)	400	400
	Cost (USD)	38,800	38,800
Feeding medium 2	Unit Price (USD/L)	70	70
	Quantity (L)	40	40
	Cost (USD)	2,800	2,800

Item	Traditional fedbatch	High dilution perfusion
Total Price per Batch (USD)	61,340	108,000
Expression Level (g/L)	5.43	9.06
Batch Yield (g)	10,860	18,120
Cost per Gram of Protein (USD)	5.6	6.0
Number of Batches per Year	18	24

Seed Train Savings: N-1 high seed perfusion offers significant savings in the seed train compared to conventional fed-batch processes, although not as substantial as N-1 high dilution perfusion. 1000L scale N-1 Perfusion can be performed in the 2000L production bioreactor.
Reduced Production Duration: Utilizing N-1 high seed perfusion can decrease production culture days from the typical 14 days in conventional fed-batch to a range of 9-12 days. This reduction in production duration contributes to overall process efficiency, faster product delivery and enhances manufacturing batch throughput.
Increased Product Titer (g/L): N-1 high seed perfusion demonstrates the capability to achieve nearly double the production titer (g/L) compared to the 14 fed-batch process. This significant increase in product yield per unit volume enhances productivity and output.
Basal Medium Consumption Considerations: It's important to note that N-1 high seed perfusion requires higher consumption of basal medium during the seed train stage compared to fed-batch processes. The cost of the basal medium needs to be factored in, as it can impact the overall cost per gram of product. However, despite this higher consumption, N-1 perfusion can still offer cost savings through reduced seed train time and production days.
In summary, N-1 high seed perfusion presents several advantages including reduced seed train requirements, shorter production durations, increased product titers, and potential cost savings despite higher basal medium consumption. These benefits contribute to improved efficiency and economic viability in biopharmaceutical manufacturing processes.

Conclusion:
Regarding N-1 high dilution perfusion, N-1 high seed perfusion, and their comparison with conventional fed-batch processes, along with the importance of selecting a suitable culture medium vendor:

1. N-1 High Dilution Perfusion Conclusion:
- N-1 high dilution perfusion offers substantial benefits in terms of seed train saving
- The process allows for faster production, improved facility utilization.
- Cost analysis reveals that while material costs may not differ significantly from traditional fed-batch processes, considerations of labor and equipment operation costs demonstrate a cost-effective advantage for the high dilution approach.
- It is crucial to select a culture medium vendor that offers competitive pricing, as the cost of each gram of product can be impacted by the choice of culture medium.

2. N-1 High Seed Perfusion Conclusion:
- N-1 high seed perfusion also presents notable advantages such as reduced seed train requirements, shorter production durations, and increased product titers close to twice that of conventional fed-batch processes.
- While there is higher consumption of basal medium during the seed train stage, overall cost savings can be achieved through reduced seed train time and production days.
- Similar to high dilution perfusion, the selection of a culture medium vendor is crucial in optimizing costs and ensuring economic viability.

3. Comparison with Conventional Fed-Batch Processes:
- Both N-1 high dilution perfusion and N-1 high seed perfusion outperform conventional fed-batch processes in terms of efficiency, productivity, and cost-effectiveness.
- The choice between the two N-1 perfusion strategies depends on specific requirements such as seed train management, production duration, and desired product titers.
- The selection of a culture medium vendor plays a vital role in determining the overall cost per gram of product, highlighting the importance of vendor assessment and cost analysis in biopharmaceutical manufacturing.

4. Scale-Up Considerations and Challenges with Larger ATF Systems:
- Scaling up N-1 perfusion culture, particularly with larger-scale ATF (Alternating Tangential Flow) systems, is essential for meeting production demands.
- However, it's important to note that as the scale of the ATF system increases, certain challenges may arise, particularly with large hollow fiber systems.
- Issues such as membrane fouling, uneven flow distribution, and potential blotting problems can occur in larger-scale hollow fiber systems, impacting overall process efficiency and productivity.
- Therefore, careful attention and optimization strategies are necessary to mitigate these challenges and ensure smooth operation of larger ATF systems in N-1 perfusion culture.

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